First Name / Last name: Paola B. Arimondo
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Unit: EpiCBio Epigenetic Chemical Biology
IP Department: Dpt of Structural Biology and Chemistry

Main domains 1: epigenetics, drug design, chemical biology

Main domain 2: chemistry, biochemistry, drug discovery

Attractive synopsis: We develop chemical compounds targeting the epigenetic regulation to reprogram cells: infected cells, cancer cells, parasites, bacteria and virus or their host.

Research projects in relation with AMR (non confidential):

EpiCBio focus on

(1) the design of chemical molecules targeting DNA and histone methylation,
(2) their use **as probes to scan the molecular process** that deregulate these modifications in human infections and
(3) their use **as potential therapeutic agents** to reprogram gene regulation in the host-pathogen interactions.

The rational is based on the fact that epigenetic changes confer to parasites the phenotypic plasticity, which characterise their progression through the different stages and environments of their life cycle. In parallel, bacterial pathogens and viruses alter the epigenetic modifications of the host. A precise elucidation of why methylation processes are aberrant in human diseases is the key to a better understanding of the disease and to fight it. The understanding of these processes will open the way to the discovery of **novel targets**, eventually also biomarkers, and **innovative therapeutic strategies**.

3 Publications

1/ Nardella, F.; Halby, L.; Hammam, E.; Erdmann, D.; Cadet-Daniel, V.; Peronet, R.; Ménard, D.; Witkowski, B.; Mecheri, S.; Scherf, A.; Arimondo, P. B., *DNA Methylation Bisubstrate Inhibitors Are Fast-Acting Drugs Active against Artemisinin-Resistant Plasmodium falciparum Parasites*. ACS Central Science **2019**. doi: 10.1021/acscentsci.9b00874.

2/ Halby L, N Marechal, D Pechalrieu, V Cura, D-M Franchini, C Faux, F Alby, N Troffer-Charlier, S Kudithipudi, A Jeltsch, W Aouadi, E Decroly, J-C Guillemot, P Page, C Ferroud, L Bonnefond, D Guianvarc'h, J Cavarelli and PB Arimondo *Hijacking DNA methyltransferase transition state analogues to produce chemical scaffolds for PRMT inhibitors* Phil. Trans RS B, **2018**, 373(1748) pii: 20170072.

3/ Halby L, Menon Y, Rilova E, Pechalrieu D, Masson V, Faux C, Bouhlel MA, David-Cordonnier MH, Novosad N, Aussagues Y, Samson A, Lacroix L, Ausseil F, Fleury L, Guianvarc'h D, Ferroud C, Arimondo PB. *Rational Design of Bisubstrate-Type Analogues as Inhibitors of DNA Methyltransferases in Cancer Cells.* J Med Chem. **2017** 60(11):4665-4679.

First Name / Last name: Rayan CHIKHI
Contact: rayan.chikhi@pasteur.fr

Unit: Sequence Bioinformatics (G5)
IP Department or IP: Computational Biology

Main domain 1: Bacteria, Mammals

Main domain 2: biomarkers and diagnostic, technological and methodological developments.

Attractive synopsis: Development of bioinformatics algorithms and methods, applied to biological problems, e.g. detection of AMR genes

Research projects in relation with AMR:
We develop bioinformatics methods for the analysis of large-scale sequencing datasets, whole transcriptomes, and whole genomes. The group has extensive experience on de novo assembly of bacterial genomes, a key preliminary step in AMR projects. We developed one of the fastest and most accurate assembly methods to date (Minia assembler), which is also capable of assembling metagenomes. In addition, a highlight from last year is our DE-Kupl software (citation below), capable of detecting diverse classes of variants (not just SNPs) in human transcriptomes, associated to a certain condition. In principle, this method can be applied to bacterial genomes and AMR. Another of our ongoing projects is relative to the indexing of thousands of sequencing datasets. Biological investigators will be able to quickly search a collection of sequencing datasets for the presence of a variant, i.e. BLAST-ing raw data instead of genomes, which bypasses the need to assemble data.

3 Publications

- The Computational Pan-Genomics Consortium, *Computational pan-genomics: status, promises and challenges*, Briefings in Bioinformatics (2016)
- C. Sun, R. S. Harris, R. Chikhi, P. Medvedev, *AllSome Sequence Bloom Trees*, RECOMB (2017)
- J. Audoux et al., *DE-kupl: exhaustive capture of biological variation in RNA-seq data through k-mer decomposition*, Genome Biology (2017)

First Name / Last name: HAOUZ Ahmed
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Unit: PF Crystallography
IP Department: C2RT, Structural Biology and Chemistry

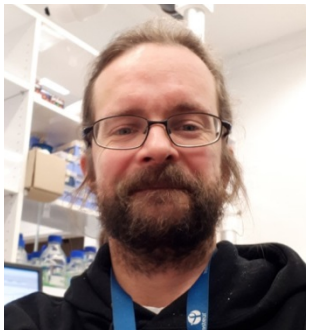
Attractive synopsis:
Crystallography is a powerful technique used in the development of antimicrobial drugs.

Main domains: structural biology, X-ray crystallography

Research projects in relation with AMR:
During the last ten years, we have been involved in a number of projects related to AMR. These projects are focused on the ligases [1,2], lactamases[3], and transglycosylases [4] involved in the synthesis of bacterial cell walls.
Recently by exploiting the protein functional motions of *T. Cruzi* proline racemase as a therapeutic target, we successfully designed new inhibitors against Chagas disease [5].
Human IMPDHs are important targets for immunosuppressive cancer and antiviral chemotherapy. Our recent work has revealed the crucial role of one of the two structural domains (the Bateman domain composed of two CBS domains) in the MgATP-dependent allosteric and oligomeric state regulation of bacterial IMPDHs. We took advantage of these novel properties to search for allosteric inhibitors of the *Pseudomonas aeruginosa* IMPDH. For this purpose, different chemical libraries were screened and several inhibitors have been identified. Their modes of action were deciphered by biochemical and biophysical approaches. In particular, competitive inhibitors for the natural positive effector Mg-ATP were selected and confirmed to be promising potential allosteric inhibitors. Our structural studies by SAXS and X-ray Crystallography revealed an original binding mode which traps the protein in its low affinity apo-conformation for IMP[6]. This work paves the way for future development of novel antimicrobials targeting IMPDHs from major bacterial pathogens such as *Pseudomonas aeruginosa*.

Publications

1- Meziane-Cherif D, Bonnet R, **Haouz A**, Courvalin P. “Structural insights into the loss of penicillinase and the gain of ceftazidimase activities by OXA-145 β-lactamase in Pseudomonas aeruginosa. ” **J Antimicrob Chemother**. 2016 Feb;71(2):395-402.
2- Williams AH, Wheeler R, Rateau L, Malosse C, Chamot-Rooke J, **Haouz A**, Taha MK, Boneca IG. “A step-by-step in crystallo guide to bond cleavage and 1,6-anhydro-sugar product synthesis by a peptidoglycan-degrading lytic transglycosylase. ” **J Biol Chem**. 2018 Apr 20;293(16):6000-6010.
3- Amaral PA, Autheman D, de Melo GD, Gouault N, Cupif JF, Goyard S, Dutra P, Coatnoan N, Cosson A, Monet D, **Saul F**, **Haouz A**, Uriac P, Blondel A, Minoprio P. “Designed mono- and di-covalent inhibitors trap modeled functional motions for Trypanosoma cruzi proline racemase in crystallography. ” **PLoS Negl Trop Dis**. 2018 Oct 29;12(10):e0006853.



First Name / Last name: Marcel Hollenstein
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Unit: Bioorganic Chemistry of nucleic acids (BCNA)
IP Department or IP: Structural Biology and Chemistry
Secondary affiliation: Genome and Genetics

Main domain 1: Bacteria

Main domain 2: biomarkers and diagnostic, new molecules, alternative strategies, and technological and methodological developments.

Attractive synopsis:
We develop modified aptamers for the detection and selective destruction of multiresistant bacteria and stop progression of diseases.

Research projects in relation with AMR:

1. Selection of modified aptamers for a biomarker of malaria: we have selected aptamers, using a nucleoside triphosphate equipped with an exotic, hydrophobic group against the lactate dehydrogenase of P. vivax (PvLDH). The high selectivity of this aptamer is currently being exploited to develop a diagnostic test for the detection of malaria.
2. Selection of aptamers for the detection of pneumonia: we have selected aptamers, using a nucleoside triphosphate equipped with a fluorophore, for the selective recognition of a specific strain of Streptococcus pneumoniae (the Pneumococcus).
3. We are currently synthesizing other modified nucleotides to isolate other modified aptamers against important targets such as the M2 protein for the treatment of Influenza A virus.
4. We develop nucleic acid based tools for gene silencing activities and photodynamic therapy.

3 (max) Publications

- Röthlisberger, P.; Hollenstein, M.*, Aptamer Chemistry, *Adv. Drug Deliv. Rev.*, 2018, *134*, 3-21
- Thai, H. B. D.; Levi-Acobas, F.; Yum, S.-Y.; Jang, G.; Hollenstein, M.; Ahn, D.-R.*, Tetrahedral DNAzymes for enhanced intracellular gene-silencing activity, 2018, *Chem. Commun.*, 2018, *54*, 9410-9413
- Röthlisberger, P.; Levi-Acobas, F.; Sarac, I.; Baron, B.; England, P.; Marlière, P.; Herdewijn, P.; Hollenstein, M.*, Facile immobilization of DNA using an enzymatic his-tag mimic, *Chem. Commun.*, 2017, *53*, 13031-13034

First Name / Last name: Nadia Izadi-Pruneyre
Contact : nadia.izadi@pasteur.fr

Unit: Structural Bioinformatics
IP Department or IP: Structural Biology and Chemistry

Main domains 1: Bacteria, Fungi, Insects, Parasites or Virus
Bacteria
Main domain 2: surveillance and epidemiology, mechanism of resistance and dissemination, biomarkers and diagnostic, new molecules, alternative strategies, technological and methodological developments.
New molecules, Technological and methodological developments,

Attractive synopsis:
We recently developed a new approach that combines NMR in whole cell and in silico docking to characterise hit-target interactions at the atomic level.

Research projects in relation with AMR:
Detailed information on hit-target interaction is crucial for drug discovery programs. We developed a new approach combining NMR in whole-cells (***in-cell*** **NMR**) and **in silico docking** to characterize hit-target interaction at the atomic level. We have recently used this method to decipher the binding mode of promising **antituberculosis drugs** with their target. The drugs had been identified by phenotypic screening by Institut Pasteur of Korea, and their most advanced (Q203) is currently in clinical trial. The target, cytochrome bc1, was identified by genome sequencing of resistant mutants. By using in cell NMR, we studied drug interactions with living bacterial cells expressing wild type and mutant cytochrome bc1, and identified the atoms involved in this interaction. This atomic information will be used for **lead optimisation**. This new approach will be highly valuable for target engagement validation and lead optimisation.

3 Publications (related to the research project described above)

- Target Engagement and Binding Mode of an Antituberculosis Drug to Its Bacterial Target Deciphered in Whole Living Cells by NMR. Bouvier G, Simenel C, Jang J, Kalia NP, Choi I, Nilges M, Pethe K, Izadi-Pruneyre N. Biochemistry. 2019 Feb 12;58(6):526-533. doi: 10.1021/acs.biochem.8b00975

- International patent: Docking method based on saturation transfer difference NMR data, and means for its implementation WO 2019/011987.

First Name / Last name: Malliavin Thérèse
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Unit: Structural Bioinformatics
IP Department or IP: Structural Biology and Chemistry
Secondary affiliation: Center of Bioinformatics, Biostatistics and Integrative Biology

Main domains 1: Bacteria

Main domain 2: new molecules, technological and methodological developments.

Attractive synopsis:
I use molecular modeling for analyzing functional dynamics of proteins having relation to the microbial resistance and microbial virulence.

Research projects in relation with AMR (non confidential):
I have been studying, in collaboration with Patrick Courvalin (Agents antibactériens, Institut Pasteur) the functional dynamics of VanA, a D-Ala:D-Lac ligase playing a key role in the emergence of high level resistance to vancomycin in *Enterococcus* species and Methicillin-Resistant *Staphylococcus aureus*. I am now studying in collaboration with Emmanuel Lemichez (Toxines Bactériennes, Institut Pasteur) the *Escherichia coli* Cytotoxic Necrotizing Factor 1 (CNF1), a bacterial toxin having specific enzymatic activity on pivotal molecular switches in eukaryotic cells. In both cases, I use molecular dynamics simulations to investigate the functional dynamics related to the accessibility of the catalytic site of these two enzymes.

3 Publications

- Duclert-Savatier N, Bouvier G, Nilges M, Malliavin TE. Conformational sampling of CpxA: Connecting HAMP motions to the histidine kinase function. PLoS One. 2018 Nov 29;13(11):e0207899. doi: 10.1371/journal.pone.0207899. eCollection 2018.
- Duclert-Savatier N, Bouvier G, Nilges M, Malliavin TE. Building Graphs To Describe Dynamics, Kinetics, and Energetics in the d-ALa:d-Lac Ligase VanA. J Chem Inf Model. 2016 Sep 26;56(9):1762-75. doi: 10.1021/acs.jcim.6b00211. Epub 2016 Sep 12.
- Bouvier G, Duclert-Savatier N, Desdouits N, Meziane-Cherif D, Blondel A, Courvalin P, Nilges M, Malliavin TE. Functional motions modulating VanA ligand binding unraveled by self-organizing maps. J Chem Inf Model. 2014 Jan 27;54(1):289-301. doi: 10.1021/ci400354b. Epub 2014 Jan 15.

First Name / Last name: Laurence MULARD
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Unit: Chimie des Biomolécules
IP Department or IP: Structural Biology and Chemistry

Main domains 1: Our expertise is applicable to all pathogens.

Main domain 2: new molecules, alternative strategies, biomarkers and diagnostic

Attractive synopsis:
Synergizing chemical biology, medicinal chemistry, and synthesis to defeat AMR by means of tailored glycans, peptides and conjugates thereof, as molecular targets, molecular tools and chemical probes on the way to innovative therapeutics, diagnostic tools or vaccines

Research projects in relation with AMR:

Scientific expertise: **chemistry**/**chemical biology** of **carbohydrates**, **peptides**, **proteins** and **bioconjugates**
Application to **antigen design**/**optimization** and **conjugate vaccine development**
- synthetic carbohydrate-based conjugate vaccines (*Shigella*, Phase II clinical trial)
- synthetic peptide-based conjugate vaccines

Application to **the design and development of drug candidates, drug targeting systems, chemical probes, labelled compounds, biomarkers, tools for screening assays**
- **molecular diversity**: compound libraries (carbohydrate & sugar nucleotide scaffolds)
- **target-based design** (structure-based): peptides (HIV), carbohydrates (microbial polysaccharides), glycopeptides (cell wall biosynthesis)

3 Publications

- Phalipon, A.; Tanguy, M.; Grandjean, C.; Guerreiro, C.; Bélot, F.; Cohen D.; Sansonetti, P.J.; **Mulard, L. A.**, A synthetic carbohydrate-protein conjugate vaccine candidate against *Shigella flexneri* 2a infection. *J Immunol* **2009**, 182, 2241-7.
- Arien, K. K., Baleux, F., Desjardins, D., Porrot, F., Coic, Y. M., Michiels, J., Bouchemal, K., Bonnaffe, D., Bruel, T., Schwartz, O., Le Grand, R., Vanham, G., Dereuddre-Bosquet, N., and Lortat-Jacob, H. CD4-mimetic sulfopeptide conjugates display sub-nanomolar anti-HIV-1 activity and protect macaques against a SHIV162P3 vaginal challenge. *Sci Rep* **2016**, 6, 34829. doi: 10.1038/srep34829.
- García-Weber, D.; Dangeard, A. S.; Cornil, J.; Thai, L.; Rytter, H.; Zamyatina, A.; **Mulard, L. A.**; Arrieumerlou, C.; ADP-heptose is a newly identified pathogen-associated molecular pattern of *Shigella flexneri*. *EMBO Reports* **2018**, e46943. doi:10.15252/embr.201846943.

First Name / Last name: Hélène Munier-Lehmann
Contact : hmunier@pasteur.fr

Unit: Chemistry and biocatalysis (biochemistry and screening group)
IP Department or IP: Structural biology and chemistry

Main domains 1: Bacteria, fungi and viruses

Main domain 2: new molecules, alternative strategies, technological and methodological developments.

Attractive synopsis:
Explore the macromolecular assembly of nucleotide biosynthesis enzymes and search for chemical compounds as modulators of these interactions to develop new antibacterials; identification of chemical tools by HTS in the field of anti-infective agents

Research projects in relation with AMR:
Nucleotides are absolutely essential to all living cells, and metabolic pathways involved in their biosynthesis are prime targets for therapeutic drugs. Recent findings on enzymes belonging to these pathways have revealed a much more organized and tight regulation than initially thought. In particular, reversible assembly of metabolic enzymes into supramolecular complexes constitutes a novel level of regulation. Our research projects aim at deciphering the organization of nucleotide metabolism enzymes in bacteria and at unraveling potential links between these metabolic enzymes and other pathways in prokaryotes through multidisciplinary approaches involving biochemistry, structural biology, chemical library screening and genetics. Perturbing the overall nucleotide metabolism could have a drastic effect on bacterial growth and/or fitness, and consequently could offer original approaches for a more selective control of infections by bacterial pathogens.

3 Publications

- Alexandre, T., Lupan, A., Helynck, O., Vichier-Guerre, S., Dugué, L., Gelin, M., Haouz, A., Labesse, G., and Munier-Lehmann, H. (2019) First-in-class allosteric inhibitors of bacterial IMPDHs. Eur. J. Med. Chem. 167, 124-132
- Laskaris, P., Vicentefranqueira, R., Helynck, O., Jouvion, G., Calera, J. A., du Merle, L., Suzenet, F., Buron, F., de Sousa, R. A., Mansuy, D., Cavaillon, J. M., Latge, J. P., Munier-Lehmann, H., and Ibrahim-Granet, O. (2018) A Novel Polyaminocarboxylate Compound To Treat Murine Pulmonary Aspergillosis by Interfering with Zinc Metabolism. Antimicrobial Agents and Chemotherapy 62, e02510-02517
- Raj, S., Krishnan, K., Askew, D. S., Helynck, O., Suzanne, P., Lesnard, A., Rault, S., Zeidler, U., d'Enfert, C., Latge, J. P., Munier-Lehmann, H., and Saveanu, C. (2016) The Toxicity of a Novel Antifungal Compound Is Modulated by Endoplasmic Reticulum-Associated Protein Degradation Components. Antimicrobial Agents and Chemotherapy 60, 1438-1449

First Name / Last name: Sylvie Pochet
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Unit: Chemistry and Biocatalysis
IP Department or IP: Structural Biology and Chemistry
Secondary affiliation: Microbiology

Main domain 1: Bacteria, Chemistry

Main domain 2: new molecules

Attractive synopsis:
Design and synthesis of NAD kinase inhibitors as novel antibacterial agents

Research projects in relation with AMR:

Multidrug resistance is a major public health problem requiring urgent development of new antibiotics and thus identification of new bacterial targets. Our ANR consortium selected the nicotinamide adenine dinucleotide kinase (NADK) as a valuable target for drug development. Structural and functional characterization of NADK led us to decipher its original enzymatic mechanism, to identify the first low micromolar synthetic inhibitor and to synthesize the first inhibitor active on bacterial cell culture. More recently, using a structure-based approach, we designed a new lead compound showing increased activity against NADKs, and more importantly being active in a mouse model of staphylococcal infection (unpublished results). We are currently working on the validation of its mode of action, as well as the development of compounds with enhanced *in vivo* activity and/or better pharmacokinetic properties.

3 Publications + 1 Brevet

1. Pochet, S.; Labesse, G.; Gelin, M.; Assairi, L.; Dussurget, O.; Poncet-Montange, G. Novel antibacterial compounds, Patent EP20100290679, WO2012090136A1.
2. Paoletti, J.; Assairi, L.; Gelin, M.; Huteau, V.; Nahori, M. A.; Dussurget, O.; Labesse, G.; Pochet, S., 8-Thioalkyl-adenosine derivatives inhibit *Listeria monocytogenes* NAD kinase through a novel binding mode. *Eur. J. Med. Chem.,* 2016, *124*, 1041-1056.
3. Gelin, M.; Poncet-Montange, G.; Assairi, L.; Morellato, L.; Huteau, V.; Dugue, L.; Dussurget, O.; Pochet, S.; Labesse, G., Screening and In Situ Synthesis Using Crystals of a NAD Kinase Lead to a Potent Antistaphylococcal Compound. *Structure,* 2012, *20*, 1107-1117.
4. Poncet-Montange G, Assairi L, Arold S, Pochet S, Labesse G. NAD kinases use substrate-assisted catalysis for specific recognition of NAD. *J. Biol. Chem.* 2007*,* 282, 33925–34